(54) Title: DESENSITIZATION TO SPECIFIC ALLERGENS

(57) Abstract

A method for desensitizing an animal to a particular allergen, wherein at or about a time of exposure of the animal to the allergen, a molecule is administered to the animal, which molecule is characterized in that it specifically binds under physiological conditions to an interleukin-4 (IL-4) receptor expressed on a peripheral blood mononuclear cell (PBMC) of the animal, and is capable of decreasing the viability of the PBMC to which it binds.

- 1 -

DESENSITIZATION TO SPECIFIC ALLERGENS
Background of the Invention

The field of the invention is prevention and
5 treatment of allergies.

An allergy is an immunological reaction, generally
of the immediate hypersensitivity type, to a particular
type of antigen termed an allergen. Such reactions
underlie attacks of anaphylaxis, allerigic rhinitis (hay
10 fever), hives, and allergic asthma, and may be triggered
by common allergens such as ragweed, pollen, bee or wasp
venom, animal dander, mold, or a component of house dust
(such as mites). In humans, immediate hypersensitivity
(IH) is mediated by antibodies of the IgE isotype
15 anchored to the surfaces of mast cells and basophils in
the skin and elsewhere. Binding of antigen to these
cell-bound IgE molecules triggers release of mediators
such as histamine from the cells, which mediators induce
the clinical phenomena such as tissue swelling, itching,
20 or bronchial smooth muscle contraction that typify an
allergic reaction.

IgE antibodies specific for a given allergen are
produced and secreted by B lymphocytes upon contact with
that allergen. Initially, B lymphocytes (or B cells)
25 express antibodies of the IgM isotype, with each B cell
committed to producing antibody specific for a particular
antigenic determinant. Contact with both an allergen
bearing that antigenic determinant, and certain factors
produced by T lymphocytes, will induce the B cell to
30 undergo what is termed an antibody heavy chain class
switch, in which the antigen-specific portion of the
antibody produced by the B cell remains the same, but it
is attached to ϵ-heavy chain (to yield IgE antibody)
rather than the μ-heavy chain of the IgM isotype. Such a
35 class switch is apparently permanent for a given B cell,

- 2 -

which thereafter secretes IgE antibody specific for the
allergen whenever stimulated to do so. One of the
factors which has been shown to be involved in this class
switch event is interleukin-4 (IL-4) (Lebman and Coffman,
5 *J. Exp. Med.* 168:853, 1988), a 20kD protein produced by T
lymphocytes. Human IL-4 has been cloned and sequenced by
Yodota et al. (*Proc. Natl. Acad. Sci. USA 83: 58994,
1986*).

          Common treatments for allergy include avoidance of
10 the suspected allergen; injections of the allergen as
immunotherapy to stimulate certain protective mechanisms
and thereby eventually desensitize the host to the
allergen; drugs such as corticosteroids, which interfere
with the release of the mediators of allergy from mast
15 cells; and drugs such as antihistamines, which block the
biological action of the released mediators.

                    Summary of the Invention
          It has now been found that, by treating B cells
which have not yet undergone the class switch from IgM
20 production to IgE production with a cytotoxic compound
(such as the recombinant protein referred to as DAB$_{389}$IL-
4) that binds to IL-4 receptors on such cells, the
production of IgE by such cells following exposure to an
allergen analog (αCD40 MAb) can be prevented. In
25 contrast, production of IgE by B cells that have
previously switched isotypes is only slightly inhibited
by treatment with such an IL-4R-targeting cytotoxic
compound, and only by very high levels of the compound.
It is believed that the mechanism by which this occurs is
30 as follows: those IgM-expressing B cells which are
stimulated by allergen to begin the process of class
switching first express a temporarily increased number of
IL-4 receptors on their surfaces, which render the cells
more likely to bind molecules of an IL-4R-targeted

- 3 -

cytotoxin. Like IL-4 itself, the IL-4R-targeted
cytotoxin causes the receptor to which it binds to be
internalized by the cell, carrying the cytotoxin, along
with the receptor to which it is bound, into the vesicle

5 so formed. The cytotoxic portion of the compound then
exits the vesicle and enters the cytoplasm of the cell,
where it enzymatically inactivates a crucial cellular
protein synthesis factor. With protein synthesis shut
off, IgE cannot be made, nor will the cell survive long:

10 thus, any B cells induced by an allergen to undergo a
class switch are selectively disabled or killed, leaving
only previously-switched B cells to produce IgE in
response to the allergen. Furthermore, treatment with a
cytotoxin that targets receptors such as IL-4R, IL-2R, or

15 IL-6R provides another mechanism of reducing IgE
production, by killing or disabling those peripheral
blood mononuclear cells (PBMC's) which are activated in
the presence of the allergen to produce factors such as
cytokines which stimulate IgE production by B cells. The

20 invention therefore features a method for desensitizing
an animal to a particular allergen, whereby   an animal
in need of such desensitization is first identified, and
at or about a time of exposure of the animal to the
allergen, a molecule is administered to the animal which

25 specifically binds under physiological conditions to an
IL-4 receptor expressed on a PBMC (preferably a B cell),
of the animal, the molecule being capable of decreasing
the viability of the PBMC (i.e., the molecule is
inherently able to contribute to the death or temporary

30 disablement of a PBMC to which it binds via an IL-4
receptor): preferably, the molecule, following binding
to an IL-4 receptor, kills the PBMC to which it has
bound. The "exposure" referred to may be a result of
deliberately administering the allergen to the patient

35 (e.g., by injection), or the patient's having

- 4 -

inadvertantly or intentionally come in contact with an
environmental source of the allergen outside of a
clinical setting (e.g., by inhaling ragweed during hay
fever season, by holding a pet the dander of which is
5   allergenic, or by receiving a bee sting). To be
effective, the IL-4R-targeted cytotoxin must be
administered at or about the time of (i.e., just prior
to, contemporaneously with, or soon after) exposure of
the subject animal to the allergen of interest, to ensure
10 that most or all of the animal's allergen-stimulated,
unswitched B cells and other PBMCs will bear their
temporarily heightened levels of IL-4 receptors during
the period that the cytotoxin is present in the animal's
bloodstream. Administration of the cytotoxin may be
15 continued even after contact with the allergen has
ceased, to ensure that all susceptible B cells are
ultimately prevented from making the isotype switch,
and/or that other IL-4R-bearing PBMCs in the animal are
prevented from contributing to the IgE-secretion process.
20 Because at any time new B cells may arise which have the
potential of being triggered into switching isotype by
the allergen, the treatment is preferably repeated on a
regular basis.

        The animal is preferably a mammal such as a mouse
25 or a dog, and most preferably is a human patient who is
either naive (i.e., has never previously been exposed to
the allergen of interest, or at least never in a manner
sufficient to trigger an immunogenic response), or is
atopic (i.e., has demonstrated an allergic response to
30 this or a related allergen in the past). Thus, the term
"desensitization" refers not only to the method as
applied to atopic individuals, but also as the method is
applied to anyone, even naive subjects, who may be
treated prophylactically in order to ensure they never
35 develop an allergy to the given allergen. Such

- 5 -

prophylactic treatment would be of particular benefit to
children of atopic parents, who run a greatly increased
risk, compared to the children of non-atopic parents, of
eventually developing allergies.

5   In preferred embodiments, the molecule used in the
method of the invention is a hybrid molecule (such as a
polypeptide) having a first and a second portion joined
together covalently, the first portion including a moiety
capable of decreasing cell viability and the second

10 portion including a moiety capable of specifically
binding to an IL-4 receptor on a PBMC under physiological
conditions [i.e., upon contact with such an IL-4 receptor
under physiological conditions, the moiety binds to IL-4
receptors and does not bind detectably to any other

15 structure found on the surfaces of PBMC's from the same
species]. By "under physiological conditions" is meant
in blood or serum, or in an aqueous solution such as
phosphate-buffered saline that approximates the pH and
salt conditions which occur in blood *in vivo*. The IL-4R-

20 binding moiety may be, for example, IL-4, an IL-4R-
binding portion of IL-4, an IL-4R-binding monoclonal
antibody, or an IL-4R-binding portion of such a
monoclonal antibody. Where the animal to be treated is a
human, the moiety is preferably human IL-4.

25   The "first portion" of the hybrid molecule
preferably includes an enzymatically-active segment of a
polypeptide toxin such as diphtheria toxin, *Pseudomonas*
exotoxin A, ricin, Shiga toxin, Shiga-like toxin-I,
Shiga-like toxin II, Shiga-like toxin II$_v$, *E. coli* LT,

30 *Salmonella* LT, cholera toxin, C3 toxin, pertussis toxin,
tetanus toxin, abrin, modeccin, volkensin, viscumin,
alorin, saporin, or gelonin, and more preferably includes
fragment A of diphtheria toxin and a portion of fragment
B of diphtheria toxin, but does not possess a generalized

35 eukaryotic cell-binding activity such as is found on

- 6 -

fragment B of diphtheria toxin and many other toxins.
Most preferably, the toxin segment is DAB_{389} or DAB_{486},
and the hybrid molecule is $DAB_{389}IL-4$ or $DAB_{486}IL-4$.
Treatment with such an IL-4R-binding cytotoxin may
5   optionally be accompanied by administering to the animal
a second cytotoxic molecule which specifically binds
under physiological conditions to an interleukin-2 (IL-2)
or interleukin-6 (IL-6) receptor on the same PBMC as is
bound by the IL-4R-binding cytotoxin, or on a different
10 PBMC (monocyte, T cell or B cell). Such ancillary
treatment may help minimize the level of IgE production
resulting from contact with the allergen.

        Also within the invention is a method for
inhibiting the antibody heavy-chain class switching of a
15 B cell by treating a B cell which has not yet undergone
class switching with a molecule which is capable of
specifically binding to an IL-4 receptor expressed on the
surface of the B cell, the molecule being capable of
decreasing the viability of the B cell (i.e., the
20 molecule possesses the inherent capacity to contribute to
the disablement or death of a B cell to which it binds
via an IL-4 receptor). This method may be carried out *in
vitro*, using a biological sample such as blood or
purified B cells, or *in vivo*, such as in a human patient.
25 It is preferentially accompanied by the additional step
of contacting the B cell with an allergen at or about the
same time as the treatment step (i.e., shortly before,
during, or soon after the treatment step). The molecule
used may be any of the cytotoxic IL-4R-binding hybrid
30 molecules discussed herein, but is preferably a
diphtheria toxin-based recombinant polypeptide such as
$DAB_{389}IL-4$.

        By selectively killing or disabling B cells poised
to switch to the IgE isotype upon contact with a given
35 allergen, the method of the invention provides an

- 7 -

effective means of desensitizing individuals to that
allergen. For those who suffer from allergies,
preventing such class switching may result in a gradually
decreased allergic response to the allergen as existing

5   IgE and IgE-producing cells are naturally turned over
without being replaced by newly-switched B cells. Naive
individuals desensitized to a given allergen in
accordance with the method of the invention may never
develop an allergy to that allergen.

10   Other features and advantages of the invention
will be apparent from the following detailed description,
and from the claims.

Detailed Description

The drawing is first described.

15 ### Drawing

Fig. 1 is a representation of the amino acid
sequence of $DAB_{389}IL-4$ and a DNA sequence encoding this
hybrid protein (SEQ ID NO: 1).

IL-4-receptor-targeted toxins

20   The compounds useful in the method of the
invention preferably contain toxic moieties, such as
bacterial polypeptide toxins or enzymatically-active
portions thereof, which are significantly cytotoxic only
when present intracellularly. Of course, under these

25 circumstances, the molecule must be able to enter a cell
bearing the targeted IL-4 receptor (IL-4R). This may be
accomplished by including on the toxin molecule a ligand
(such as IL-4 itself, or a portion of IL-4 capable of
binding to the IL-4 receptor, or an anti-IL-4R antibody)

30 which, upon binding to the receptor, induces the
internalization of the receptor and anything bound to it.
Such an IL-4R-binding moiety can be linked to the toxin
molecule chemically, using standard chemical conjugation

- 8 -

techniques. Alternatively, the linkage can be
accomplished by engineering a hybrid recombinant DNA
molecule which encodes both the IL-4R-binding moiety and
the toxin in a single polypeptide. The latter approach
5 ensures consistency of composition.

Many peptide toxins have a generalized eukaryotic
receptor binding domain; in these instances the toxin
must be modified to prevent intoxication of non-IL-4R-
bearing cells. Any such modifications must be made in a
10 manner which preserves the cytotoxic functions of the
molecule. Potentially useful polypeptide toxins include,
but are not limited to: diphtheria toxin, *Pseudomonas*
exotoxin A, cholera toxin, ricin, Shiga toxin, the Shiga-
like toxins (SLT-I, SLT-II, SLT II_V), *E. coli* LT,
15 *Salmonella* LT, C3 toxin, pertussis toxin, tetanus toxin,
abrin, modeccin, volkensin, viscumin, alorin, saporin,
and gelonin.

Other types of toxic moieties which may be linked
to an IL-4R-binding ligand for use in the method of the
20 invention include, for example, radionuclides and cancer
chemotherapeutic agents.

Diphtheria Toxin-based Molecules

Diphtheria toxin, which is described in detail in
Murphy U.S. Patent No. 4,675,382 (hereby incorporated by
25 reference), can be used to produce molecules useful in
the method of the invention. The natural diphtheria
toxin molecule secreted by *Corynebacterium diphtheriae*
consists of several functional domains which can be
characterized, starting at the amino terminal end of the
30 molecule, as fragment A (amino acids Gly_1 - Arg_{193}), which
is the enzymatically-active portion of the protein, and
fragment B (amino acids Ser_{194} - Ser_{535}), which includes a
translocation domain and a generalized cell binding
domain (amino acid residues 475 through 535).

The process by which diphtheria toxin intoxicates sensitive eukaryotic cells involves at least the following steps: (i) the binding domain of diphtheria toxin binds to specific receptors on the surface of a

5   sensitive cell; (ii) while bound to its receptor, the toxin molecule is internalized into an endocytic vesicle; (iii) either prior to internalization, or within the endocytic vesicle, the toxin molecule undergoes a proteolytic cleavage between fragments A and B; (iv) as

10   the pH of the endocytic vesicle decreases to below 6, the toxin crosses the endosomal membrane, facilitating the delivery of fragment A into the cytosol; (v) the catalytic activity of fragment A (i.e., the nicotinamide adenine dinucleotide - dependent adenosine diphosphate

15   (ADP) ribosylation of the eukaryotic protein synthesis factor termed "Elongation Factor 2") causes the death of the intoxicated cell. It has been shown that a single molecule of fragment A introduced into the cytosol is sufficient to shut down the cell's protein synthesis

20   machinery, thereby killing the cell. The mechanism of cell killing by *Pseudomonas* exotoxin A, and possibly by certain other naturally-occurring toxins, is the same.

        $DAB_{389}IL-4$, a genetically engineered fusion protein in which the receptor binding domain of

25   diphtheria toxin has been replaced by human IL-4, is an example of a molecule useful in the method of the invention. This molecule selectively kills IL-4R-expressing cells, including lymphocytes and certain tumor cells. $DAB_{389}IL-4$ is a chimeric molecule consisting of

30   (from the amino to the carboxy terminus) Met followed by amino acid residues 1 through 386 of mature diphtheria toxin, followed by a His-Ala dipeptide, followed by all of the amino acid residues of IL-4. Thus, $DAB_{389}IL-4$ includes all of diphtheria toxin fragment A (the

35   enzymatically active portion of the molecule), and a

- 10 -

portion of fragment B. The portion of fragment B present
in DAB$_{389}$IL-4 does not include the generalized receptor
binding domain of diphtheria toxin, but does include the
translocation domain which facilitates delivery of the
5 enzymatically active portion into the cytosol.

Preparation of DAB$_{389}$IL-4

        A synthetic gene encoding human interleukin-4 was
synthesized (Milligen/Biosearch 7500 DNA synthesizer).
The IL-4 sequence (Yodota et al., *Proc Nat'l Acad Sci.*
10 *USA*, 83:58994, 1986) was modified to incorporate E. coli-
preferred codon usage (deBoer et al., in *Maximizing Gene
Expression*, Reznikioff et al., eds., 1986, Butterworths,
Boston), and restriction endonuclease cleavage sites were
added to facilitate subsequent cloning steps. IL-4
15 coding sequence (His^1 to Ser^{129}) was inserted into pSE5
plasmid (Shaw et al., J. Biol. Chem. 266:21118, 1991).
The DNA sequence and corresponding amino acid sequence of
this hybrid gene are shown in Fig. 1 (SEQ ID NO: 1).
Following expression of DAB$_{389}$IL-4 in *E.coli*, the fusion
20 protein was purified by standard techniques.

        Alternatively, the portion of diphtheria toxin
utilized in the hybrid toxin can be longer or shorter
than DAB$_{389}$, provided that the portion used contains the
enzymatically active domain and the translocation domain
25 of diphtheria toxin, and does not contain a functional
generalized eukaryotic cell-binding domain of the
naturally-occurring toxin. For example, a portion
containing amino acids 1 to 485 of diphtheria toxin has
been incorporated into certain toxin hybrids (where the
30 cell-binding function is supplied by a ligand such as IL-
2 or α-MSH), and the resulting hybrid polypeptide has
been found to intoxicate and kill cells bearing receptors
for that ligand. It would therefore be expected that
this 485-amino acid segment of diphtheria toxin, as well

- 11 -

as many others containing longer or shorter portions of
fragment B sequence, would provide the necessary
functions of diphtheria toxin without the undesired
generalized cell-binding function of the naturally-
occurring toxin molecule.

Yet another strategy for preparing the toxin
portion of the hybrid would be to inactivate the
receptor-binding domain of diphtheria toxin by, for
example, making point mutations or internal deletions
within this domain that inhibit the toxin's ability to
bind to its natural receptor (Greenfield et al., *Science*
238:536, 1987).

Other Toxins

The cytotoxic portion of hybrid molecules useful
in the invention can alternatively be provided by another
type of toxin molecule. For example, hybrid toxins
containing the enzymatically-active and translocation
domains of *Pseudomonas* exotoxin A linked to IL-4 (or
another IL-4R-binding ligand) can be produced by
recombinant techniques in a manner analogous to that
described by Chaudhary et al. (*Proc. Natl. Acad. Sci. USA*
84:4538-4542, 1987) for a *Pseudomonas* exotoxin A/TGF-α
hybrid. The cell-binding regions of other toxins,
including ricin, cholera toxin, *E. coli* LT, *Salmonella*
LT, Shiga toxin, the Shiga-like toxins, abrin, modeccin,
volkensin, and viscumin, have been shown to be located on
subunits separate from those bearing the enzymatically-
active or effector regions of these molecules, and so can
be deleted from the toxin either by such standard means
as genetic engineering or reduction of the disulfide
bonds linking the subunits of a given toxin. Some of
these toxins (cholera toxin and the LTs) do not typically
kill the cell they intoxicate, but rather disable the
cell temporarily by interfering with normal regulation of

- 12 -

cyclic adenosine monophosphate (cAMP) production. Thus,
the use in the method of the invention of hybrid
molecules that employ the effector regions of these
particular toxins may be of benefit where temporary
5 disablement, rather than killing of the target B cell, is
desired.

The DNA and/or amino acid sequences corresponding
to some of these naturally-occurring toxins have been
published [e.g., Shiga toxin (Strockbine et al., *J.*
10 *Bacteriol.* 170:1116-1122, 1988); SLT-II (Jackson et al.,
FEMS Microbiol. Lett. 44:109-114, 1987); cholera toxin
(Mekalanos et al., *Nature* 306:551-557, 1983); and *E. coli*
LT (Spicer and Noble, *J. Biol. Chem.* 257:5716-5721,
1982), all of which are hereby incorporated by
15 reference], and the sequences of others can be determined
by standard cloning and sequencing techniques well known
to those of ordinary skill in the art.

Another source of the toxic portion of the IL-4R-
targeted toxin is what is herein termed a "combination"
20 toxin. A combination toxin is a molecule having a
portion of its amino acid sequence derived from one
polypeptide toxin and another portion derived from a
different polypeptide toxin. The combination toxins
useful in the invention would have an enzymatically
25 active domain derived from one type of naturally-
occurring toxin, a translocation domain derived from
another type of toxin, and a functional cell-binding
domain derived from neither; the IL-rR-binding ligand
would supply the only cell-binding function of this
30 hybrid molecule.

Naturally-occurring proteins which are known to
have a translocation domain include diphtheria toxin,
Pseudomonas exotoxin A, and possibly other peptide
toxins. The translocation domains of diphtheria toxin
35 and *Pseudomonas* exotoxin A are well characterized (see,

- 13 -

e.g., Hoch et al., *Proc. Natl. Acad. Sci. USA* 82:1692,
1985; Colombatti et al., *J. Biol. Chem.* 261:3030, 1986;
and Deleers et al., *FEBS Lett.* 160:82, 1983), and the
existence and location of such a domain in other
5   molecules may be determined by methods such as those
employed by Hwang et al. *Cell* 48:129, 1987; and Gray et
al. *Proc. Natl. Acad. Sci. USA* 81:2645, 1984).

One useful IL-4/mixed toxin hybrid molecule is
formed by fusing the enzymatically active A subunit of *E.*
10   *coli* Shiga-like toxin (Calderwood et al., *Proc. Natl.*
Acad. Sci. USA 84:4364, 1987) to a portion of fragment B
of diphtheria toxin that includes a proteolytically-
sensitive disulfide loop and the translocation domain
(amino acid residues 186 through 386) of diphtheria
15   toxin, and to IL-4. This three-part hybrid molecule,
SLT-A/DTB'/IL-4, is useful in the method of the invention
in the same way as DAB$_{389}$IL-4 described above. The IL-4
portion of the three-part hybrid causes the molecule to
attach specifically to IL-4R-bearing cells, and the
20   diphtheria toxin translocation portion participates in
the insertion of the enzymatically-active A subunit of
the Shiga-like toxin into the targeted cell. The
enzymatically active portion of Shiga-like toxin, like
diphtheria toxin, acts on the protein synthesis machinery
25   of the cell to prevent protein synthesis, thus killing
the cell. The difference between these two types of
hybrid toxins is the nature of their enzymatic
activities: the enzymatic portion of DAB$_{389}$IL-4 catalyzes
the ADP-ribosylation by nicotinamide adenine dinucleotide
30   of Elongation Factor 2, thereby inactivating this factor
which is necessary for protein synthesis, while the
enzymatic portion of SLT-A/DTB'/IL-4 is a ribonuclease
capable of cleaving ribosomal RNA at a critical site,
thereby inactivating the ribosome. SLT-A/DTB'/IL-4
35   hybrid would therefore be useful as a treatment for the

- 14 -

same indications as $DAB_{389}IL-4$, and could be substituted
or used in conjunction with it if, for example, a
patient's B cells develop a resistance to $DAB_{389}IL-4$.

Other IL-4R-binding ligands

5   The hybrid toxin useful in the method of the
invention may employ as the IL-4R-binding ligand a moiety
other than full-length IL-4. By deleting various
portions of the DNA encoding IL-4 using standard genetic
engineering techniques, fragments of IL-4 are generated
10 which can be readily tested in an assay such as that
described by Waters et al. (Eur. J. Immunol. 20:485,
1990) for their ability to bind to IL-4 receptors.
Alternatively, monoclonal antibodies useful in the method
of the invention can be made by immunizing mice with
15 human IL-4R^{+} lymphocytes (e.g., using methods similar to
those of Beckmann et al., J. Immunol. 144:4212-4217,
1990), fusing the murine splenocytes with appropriate
myeloma cells, and screening the antibodies produced by
the resultant hybridoma lines for the requisite IL-4R
20 binding properties by, for example, assaying their
ability to inhibit ^{125}I-labeled IL-4 binding to IL-4R^{+}
cells using the method of Mosley et al., Cell 59:335-348,
1989. Alternatively, useful antibodies may be isolated
from a combinatorial library produced by the method of
25 Huse et al. (Science 246:1275, 1989).
          The invention can employ not only intact
monoclonal antibodies as the IL-4R-binding ligand, but
also an immunologically-active antibody fragment, for
example, a Fab or (Fab)$_2$ fragment; an antibody heavy
30 chain; an antibody light chain; a genetically engineered
single-chain Fv molecule (Ladner et al., U.S. Patent No.
4,946,778); or a chimeric antibody, for example, an
antibody which contains the binding specificity of a
murine antibody, but in which the remaining portions are

- 15 -

of human origin, or an antibody whose Fv region is
genetically engineered to capture the higher binding
affinity of its target receptor's natural targeting
ligand.

5   Linkage of Toxins to Binding Ligands
        The binding ligand and the cytotoxin of useful
hybrid molecules can be linked in several ways. If the
hybrid molecule is produced by expression of a fused
gene, a peptide bond serves as the link between the
10 cytotoxin and the binding ligand. Alternatively, the
toxin and the binding ligand can be produced separately
and later coupled by means of a non-peptide covalent
bond, such as a disulfide bond. In this case, if the
binding ligand is a protein, e.g., IL-4, the DNA encoding
15 IL-4 can be engineered to contain an extra cysteine codon
in a manner analogous to that described in Murphy et al.
U.S. Serial No. 313,599, hereby incorporated by
reference. The cysteine must be positioned so as to not
interfere with the IL-4R binding activity of the hybrid
20 molecule. For example, the cysteine codon can be
inserted just upstream of the DNA encoding the mature
form of IL-4. The toxin molecule must be derivatized
with a sulfhydryl group reactive with the cysteine on the
modified IL-4. In the case of a peptide toxin, this can
25 be accomplished by inserting an extra cysteine codon into
the DNA sequence encoding the toxin. Alternatively, a
sulfhydryl group, either by itself or as part of a
cysteine residue, can be introduced using known synthetic
techniques. For example, the introduction of sulfhydryl
30 groups into peptides is described by Hiskey (*Peptides*
3:137, 1981). Derivatization can also be carried out
according to the method described for the derivatization
of a peptide hormone in Bacha et al. U.S. Patent No.
4,468,382, hereby incorporated by reference. The

- 16 -

introduction of sulfhydryl groups into proteins is
described in Maasen et al. (*Eur. J. Biochem.* 134:32,
1983). Once the correct sulfhydryl groups are present,
the cytotoxin and IL-4R-binding ligand are separately
5 purified; both sulfur groups are reduced; cytotoxin and
ligand are mixed (in a ratio of about 1:5 to 1:20); and
disulfide bond formation is allowed to proceed to
completion (generally 20 to 30 minutes) at room
temperature. The mixture is then dialyzed against
10 phosphate buffered saline to remove unreacted ligand and
toxin molecules. Sephadex chromatography or the like is
then carried out to separate on the basis of size the
desired toxin-ligand conjugates from toxin-toxin and
ligand-ligand conjugates.

15 <u>Assays for IL-4 Receptor Binding</u>
        The IL-4R binding activity of various molecules
can be measured using the assay described by Park et al.
(*J. Exp. Med.* 166:476, 1987) or the assay described by
Foxwell et al. (*Eur. J. Immunol.* 19:1637, 1989).

20 <u>Assays for Toxicity</u>
        Toxicity towards IL-4R bearing cells in general
can be tested as follows. Cultured HUT 102/6TG cells
(Tsudo et al., *Proc. Natl. Acad. Sci. USA* 83:9694, 1986)
or MLA144 cells (Rabin et al. *J. Immunol.* 127:1852, 1981)
25 are maintained in RPMI 1640 medium (Gibco, Grand Island,
NY) supplemented with 25 mM HEPES (pH 7.4), 2mM 1-
glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin,
and 10% fetal calf serum (Hazelton, Lenexa, KS). Cells
are seeded in 96-well V-bottomed plates (Linbro-Flow
30 Laboratories, McLean, VA) at a concentration of 1 x 10^5
per well in complete medium. Putative toxins are added
at varying concentrations (10^{-12}M to 10^{-6}M) and the
cultures are incubated for 20 hrs. at 37°C in a 5% CO_2

- 17 -

atmosphere. Following incubation, the plates are
centrifuged for 5 min. at 170 x g, and the medium removed
and replaced with 100 μl leucine-free medium (MEM, Gibco)
containing 8 μCi/ml (^{3}H-leucine; New England Nuclear,
5 Boston, MA). After an additional 90 min. at 37°C, the
plates are centrifuged for 5 min. at 170 x g, the medium
is removed, and the cells are collected on glass fiber
filters using a cell harvester (Skatron, Sterling, VA).
Filters are washed, dried, and counted according to
10 standard methods. Cells cultured with medium alone serve
as the control. Effective cell killing is indicated by a
decrease in ^{3}H-leucine incorporation in test samples,
compared to control samples which do not contain the
toxin.

15 Assay for ability to prevent class-switching of B cells
Materials and Methods

Interleukins and Antibodies. Human rIL-4 was used
in a purified form (specific activity: 1.2 x 10^7 U/mg).
Anti-Leu-4 (IgG1 anti-CD3), anti-Leu-3a (IgG1 anti-CD4),
20 and Leu-2a (IgG1 anti-CD8), as well as the appropriate
isotype controls, were obtained from Becton Dickinson &
Co. (Mountain View, CA). F(ab^1)$_2$ fragments of monoclonal
antibody 626.1 (IgG$_1$ anti-CD40) were obtained as
described in Gruber et al., J. Immuno. 142:4144 (1989).
25 OKT3 (IgG2a anti-CD3) mAb was obtained from Ortho
Diagnostic Systems Inc. (Westwood, MA). mAb B1 (IgG2a
anti-CD20) was obtained from Coulter Immunology (Hialeah,
FL).

Cell Preparations. PBMC were isolated from
30 heparinized venous blood of normal nonallergic donors by
density gradient centrifugation on Ficoll-Hypaque, washed
three times in HBSS (Microbiological Associates,
Bethesda, MD) and resuspended in RPMI 1640/10% heat
inactivated FCS (HyClone Laboratories, Logan, UT)

- 18 -

supplemented with 2 mM L-glutamine, 50 μg/ml streptomycin
and 100 U/ml penicillin (complete medium). To obtain
purified B cells, T cells were removed by rosetting twice
with 2-aminoethylisothiouronium bromide (AET)-treated
5 SRBC. Further T cell depletion was obtained by two
cycles of lysis with anti-CD3 mAb + rabbit C (Pel-Freeze
Biologicals, Inc., Rogers, AR). To remove monocytes,
non-T cells in RPMI 1640/10% AB$^+$ serum were adhered twice
in plastic petri dishes. The resulting B cell
10 populations contained <6% CD14$^+$ cells and <1% CD3$^+$ cells,
as determined by immunofluorescence (IF). In addition,
these B cell preparations gave no proliferative response
to Con A or PHA (10 μg/ml), while they strongly
proliferated upon stimulation with PMA (25 ng/ml; Sigma
15 Chemical Co., St. Louis, MO) and insolubilized anti-μ
antibody (Immunobead rabbit anti-human IgM; 1μg/ml; Bio-
Rad Laboratories, Richmond, CA). Cell viability, as
assessed by trypan blue exclusion, was always >95%.

        Cell Cultures for IgE Induction. Purified B cells
20 (1.0 x 10^6 cells/ml) in complete medium were cultured at
37°C in a 5% CO$_2$ humidified atmosphere, in the presence
of rIL-4 (100 U/ml) and the various mAbs, as indicated
for each experiment in Results. After 10 d, the culture
supernatants were harvested and assessed by RIA for their
25 IgE content. Control cultures for the evaluation of
preformed IgE were set up in the presence of
cycloheximide (100 μg/ml; Sigma Chemical Co.). Net IgE
synthesis was evaluated by subtracting the IgE
concentrations detected in cycloheximide-treated cultures
30 from the IgE values found in untreated cultures.

        RIA for IgE. The assay was performed in flexible
flat-bottomed microtiter plates (Cooke Laboratory
Products, Alexandria, VA) at room temperature as
previously described (3). The wells were coated with 0.1
35 ml of a 1:1 mixture of purified anti-Fcϵ mAbs (7.12 and

- 19 -

4.15; a kind gift of A. Saxon, University of California
Los Angeles, Los Angeles, CA), 2 μg/ml in carbonate-
bicarbonate buffer, pH 9.6. After 16-h incubation, the
wells were washed, blocked with PBS/10% horse serum (HS)

5 for 2 h, and subsequently washed three times with PBS/1%
HS. 0.1 ml of culture supernatant or different dilutions
of IgE standard (Pharmacia Fine Chemicals) were then
added to the wells in triplicate and incubated for 16 h
in a humidified chamber. The wells were then washed one
10 time with PBS/1% HS containing 0.05% Tween 20, twice with
PBS/1% HS, and finally incubated with 0.1 ml of Phadebas
RAST ^{125}I-anti-human IgE (ND) (Pharmacia Fine Chemicals)
for 6 h. The wells were then washed three times with
PBS/1% HS/0.05% Tween 20 and eight times under running
15 distilled water, cut out, and counted in a gamma
spectrometer (Tracor Analytic, Elk Grove Village, IL).
The concentrations of IgE in the supernatants were read
from the standard curve. The lower limit of sensitivity
of this assay is 150 pg/ml. This assay was validated in
20 a recent multicenter collaborative assessment of the
variability of IgE measurement in cell culture
supernatants [Helm et al., J. Allergy Clin. Immunol.
77:880 (1986)].

Results

25   In the model system, purified surface-IgE-negative
B cells undergo class switch to IgE production if and
only if both rIL-4 and anti-CD40 monoclonal antibody are
included in the cultures. When cultured alone or in the
presence of <u>either</u> rIL-4 <u>or</u> anti-CD40 monoclonal
30 antibody, these B cells fail to produce IgE. As shown in
Table I, the addition of increasing concentrations of
DAB$_{389}$IL-4 ablated the IgE response in these cultures in a
dose-dependent manner. As long as DAB$_{389}$IL-4 was present
at the initiation of the culture, IgE synthesis was
35 inhibited in the presence of rIL-4 and anti-CD40

- 20 -

monoclonal antibody regardless of their order of
addition.

     Table II shows that purified B cells from an
atopic donor which have already undergone an Ig class
5 switch to IgE production require neither rIL-4 nor anti-
CD40 monoclonal antibody to maintain IgE production.
Furthermore, $DAB_{389}IL-4$ was unable significantly to
inhibit IgE production in cultures in which the class
switch had already occurred, in contrast to the marked
10 effect the hybrid toxin has on previously unswitched
cells.

- 21 -

Table I

$DAB_{389}IL-4$ Elimates IgE Secretion by B cells Undergoing Ig Class Switching

	Culture conditions	IgE (pg/ml)
5	B cells alone (1×10^6ml)	2
	rIL-4 (100 U/ml)	2
	CD40 mAb (F[ab]'$_2$, 5 μg/ml)	ND

A. $DAB_{389}IL-4$ for 24h, wash and add αCD40 mAb+rIL-4

10	1.	$DAB_{389}IL-4$ (10^{-7}M)	1	
	2.		(10^{-8}M)	2
	3.		(10^{-9}M)	2
	4.		(10^{-10}M)	421
	5.	Medium	398	

B. $DAB_{389}IL-4$ and αCD40 mAb for 24h, then add rIL-4

15	1.	$DAB_{389}IL-4$ (10^{-7}M)	2	
	2.		(10^{-8}M)	2
	3.		(10^{-9}M)	3
20	4.		(10^{-10}M)	3980
	5.	Medium	2584	

C. $DAB_{389}IL-4$, αCD40 mAb and rIL-4 added at beginning of culture

	1.	$DAB_{389}IL-4$ (10^{-7}M)	2	
25	2.		(10^{-8}M)	2
	3.		(10^{-9}M)	3
	4.		(10^{-10}M)	169
	5.	Medium	1372	

- 22 -

Table II

DAB$_{389}$IL-4 Does Not Elimate IgE Secretion by B cells from an Atopic Patient Which Hav Already Undergone an Ig Class Switch

5	Culture conditions		IgE (pg/ml)
	B cells alone (1 x 10^6ml)		34,446
	rIL-4 (100 U/ml)		30,338
	CD40 mAb (F[ab]'$_2$, 5 μg/ml)		29,119

10

A. DAB$_{389}$IL-4 for 24h, wash and add αCD40 mAb+rIL-4

1.	DAB$_{389}$IL-4 (10^{-7}M)		20,188
2.		(10^{-8}M)	27,646
3.		(10^{-9}M)	30,470
4.		(10^{-10}M)	28,659
15 5.	Medium		28,078

B. DAB$_{389}$IL-4 and αCD40 mAb for 24h, then add rIL-4

1.	DAB$_{389}$IL-4 (10^{-7}M)		14,064
2.		(10^{-8}M)	34,237
20 3.		(10^{-9}M)	37,506
4.		(10^{-10}M)	22,889
5.	Medium		21,353

C. DAB$_{389}$IL-4, αCD40 mAb, rIL-4 added at beginning of culture

25 1.	DAB$_{389}$IL-4 (10^{-7}M)		14,127
2.		(10^{-8}M)	39,436
3.		(10^{-9}M)	32,052
4.		(10^{-10}M)	27,290
5.	Medium		29,929

- 23 -

Animal models

The ability of a particular hybrid IL-4R-binding
toxin to diminish or ablate IgE production *in vivo* can be
studied in an animal model such as the mouse assay
5   utilized by Urban et al. (*Proc. Natl. Acad. Sci. USA*
88:5513-5517, 1991), or in a species (such as dogs) known
to develop allergic responses to certain allergens.

Therapy

Desensitization with the method of the invention
10   will probably be most effective if carried out on naive
subjects, or on atopic subjects who are not currently
mounting an allergic response. A typical protocol would
involve exposing the subject animal to one or more
allergens of interest (e.g., by intravenous injection)
15   simultaneously with the i.v. administration of the IL-4R-
specific toxin on day 1, followed by four more days of
treatment once per day with the IL-4R-specific toxin
alone. It is expected that a dosage regimen which
produces a serum concentration of about 10^{-10} to 10^{-7}M
20   $DAB_{389}IL-4$ (preferably 10^{-9} to 10^{-8}M) will effectively
kill most allergen-activated B cells about to undergo an
isotype class shift, without significant harm to those
cells which have fewer or no IL-4 receptors. This course
of treatment can be repeated several times to provide
25   effective therapy. Determination of the most efficacious
treatment protocol for desensitizing a subject to a
particular allergen or group of allergens using the
method of the invention is within the ability of one of
ordinary skill in pharmacology, using the disclosure
30   provided herein and standard pharmacological procedures.

Other embodiments are within the following claims.
What is claimed is:

- 24 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Seragen, Inc.

    (ii) TITLE OF INVENTION: Desensitization to Specific
                                  Allergens

    (iii) NUMBER OF SEQUENCES: 1

    (iv) CORRESPONDENCE ADDRESS:

        (A) ADDRESSEE: Fish & Richardson
        (B) STREET: 225 Franklin Street
        (C) CITY: Boston
        (D) STATE: Massachusetts
        (E) COUNTRY: U.S.A.
        (F) ZIP: 02110-2804

    (v) COMPUTER READABLE FORM:

        (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
        (B) COMPUTER: IBM PS/2 Model 50Z or 55SX
        (C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
        (D) SOFTWARE: WordPerfect (Version 5.0)

    (vi) CURRENT APPLICATION DATA:

        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (vii) PRIOR APPLICATION DATA:

        (A) APPLICATION NUMBER: 07/832,843
        (B) FILING DATE: 10 February 1992

    (viii) ATTORNEY/AGENT INFORMATION:

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        (B) REGISTRATION NUMBER: 34,819
        (C) REFERENCE/DOCKET NUMBER:00563/055WO1

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        (C) TELEX: 200154

- 25 -

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1604
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ATG GGC GCT GAT GAT GTT GTT GAT TCT TCT AAA TCT TTT GTG ATG GAA    48
Met Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val Met Glu
1               5                   10                  15

AAC TTT TCT TCG TAC CAC GGG ACT AAA CCT GGT TAT GTA GAT TCC ATT    96
Asn Phe Ser Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp Ser Ile
            20                  25                  30

CAA AAA GGT ATA CAA AAG CCA AAA TCT GGT ACA CAA GGA AAT TAT GAC   144
Gln Lys Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly Asn Tyr Asp
        35                  40                  45

GAT GAT TGG AAA GGG TTT TAT AGT ACC GAC AAT AAA TAC GAC GCT GCG   192
Asp Asp Trp Lys Gly Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala
    50                  55                  60

GGG TAC TCT GTA GAT AAT GAA AAC CCG CTC TCT GGA AAA GCT GGA GGC   240
Gly Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser Gly Lys Ala Gly Gly
65                  70                  75                  80

GTG GTC AAA GTG ACG TAT CCA GGA CTG ACG AAG GTT CTC GCA CTA AAA   288
Val Val Lys Val Thr Tyr Pro Gly Leu Thr Lys Val Leu Ala Leu Lys
                85                  90                  95

GTG GAT AAT GCC GAA ACT ATT AAG AAA GAG TTA GGT TTA AGT CTC ACT   336
Asp Asn Ala Glu Thr Ile Lys Lys Glu Leu Gly Leu Ser Leu Thr Glu
            100                 105                 110

GAA CCG TTG ATG GAG CAA GTC GGA ACG GAA GAG TTT ATC AAA AGG TTC   384
Val Pro Leu Met Glu Gln Val Gly Thr Glu Glu Phe Ile Lys Arg Phe
        115                 120                 125

GGT GAT GGT GCT TCG CGT GTA GTG CTC AGC CTT CCC TTC GCT GAG GGG   432
Gly Asp Gly Ala Ser Arg Val Val Leu Ser Leu Pro Phe Ala Glu Gly
    130                 135                 140

AGT TCT AGC GTT GAA TAT ATT AAT AAC ﹀GG GAA CAG GCG AAA GCG TTA   480
Ser Ser Ser Val Glu Tyr Ile Asn Asn ﹀rp Glu Gln Ala Lys Ala Leu
145                 150                 155                 160

AGC GTA GAA CTT GAG ATT AAT TTT GAA ACC CGT GGA AAA CGT GGC CAA   528
Ser Val Glu Leu Glu Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln
            165                 170                 175
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- 26 -

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GAT GCG ATG TAT GAG TAT ATG GCT CAA GCC TGT GCA GGA AAT CGT GTC   576
Asp Ala Met Tyr Glu Tyr Met Ala Gln Ala Cys Ala Gly Asn Arg Val
            180             185                     190

AGG CGA TCA GTA GGT AGC TCA TTG TCA TGC ATA AAT CTT GAT TGG GAT   624
Arg Arg Ser Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp
            195             200                     205

GTC ATA AGG GAT AAA ACT AAG ACA AAG ATA GAG TCT TTG AAA GAG CAT   672
Val Ile Arg Asp Lys Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His
            210             215                     220

GGC CCT ATC AAA AAT AAA ATG AGC GAA AGT CCC AAT AAA ACA GTA TCT   720
Gly Pro Ile Lys Asn Lys Met Ser Glu Ser Pro Asn Lys Thr Val Ser
225             230             235                     240

GAG GAA AAA GCT AAA CAA TAC CTA GAA GAA TTT CAT CAA ACG GCA TTA   768
Glu Glu Lys Ala Lys Gln Tyr Leu Glu Glu Phe His Gln Thr Ala Leu
            245             250                     255

GAG CAT CCT GAA TTG TCA GAA CTT AAA ACC GTT ACT GGG ACC AAT CCT   816
Glu His Pro Glu Leu Ser Glu Leu Lys Thr Val Thr Gly Thr Asn Pro
            260             265                     270

GTA TTC GCT GGG GCT AAC TAT GCG GCG TGG GCA GTA AAC GTT GCG CAA   864
Val Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln
            275             280                     285

GTT ATC GAT AGC GAA ACA GCT GAT AAT TTG GAA AAG ACA ACT GCT GCT   912
Val Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Thr Ala Ala
            290             295                     300

CTT TCG ATA CTT CCT GGT ATC GGT AGC GTA ATG GGC AAT GCA GAC GGT   960
Leu Ser Ile Leu Pro Gly Ile Gly Ser Val Met Gly Asn Ala Asp Gly
305             310             315                     320

GCC GTT CAC CAC AAT ACA GAA GAG ATA GTG GCA CAA TCA ATA GCT TTA  1008
Ala Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu
            325             330                     335

TCG TCT TTA ATG GTT GCT CAA GCT ATT CCA TTG GTA GGA GAG CTA GTT  1056
Ser Ser Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val
            340             345                     350
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- 27 -

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GAT ATT GGT TTC GCT GCA TAT AAT TTT GTA GAG AGT ATT ATC AAT TTA 1104
Asp Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn Leu
        355             360             365

TTT CAA GTA GTT CAT AAT TCG TAT AAT CGT CCC GCG TAT TCT CCG GGT 1152
Phe Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser Pro Gly
        370             375             380

CAC AAA ACG CAT GCT CAC AAA TGC GAC ATC ACC CTG CAG GAA ATC ATC 1200
His Lys Thr His Ala His Lys Cys Asp Ile Thr Leu Gln Glu Ile Ile
385             390             395             400

AAA ACT CTG AAT TCC CTG ACC GAA CAG AAA ACT CTG TGC ACC GAA CTG 1248
Lys Thr Leu Asn Ser Leu Thr Glu Gln Lys Thr Leu Cys Thr Glu Leu
            405             410             415

ACG GTA ACC GAC ATC TTC GCT GCA TCC AAA AAC ACC ACT GAA AAA GAA 1296
Thr Val Thr Asp Ile Phe Ala Ala Ser Lys Asn Thr Thr Glu Lys Glu
            420             425             430

ACC TTC TGC CGT GCA GCA ACT GTT CTG CGT CAG TTC TAC TCC CAC CAC 1344
Thr Phe Cys Arg Ala Ala Thr Val Leu Arg Gln Phe Tyr Ser His His
        435             440             445

GAA AAA GAC ACT CGC TGC CTT GGT GCT ACT GCA CAG CAG TTC CAC CGT 1392
Glu Lys Asp Thr Arg Cys Leu Gly Ala Thr Ala Gln Gln Phe His Arg
        450             455             460

CAC AAA CAG CTG ATC CGT TTC CTG AAA CGT CTA GAC CGC AAC CTG TGG 1440
His Lys Gln Leu Ile Arg Phe Leu Lys Arg Leu Asp Arg Asn Leu Trp
465             470             475             480

GGC CTG GCT GGC CTG AAC TCC TGT CCG GTT AAA GAA GCT AAC CAG TCG 1488
Gly Leu Ala Gly Leu Asn Ser Cys Pro Val Lys Glu Ala Asn Gln Ser
            485             490             495

ACC CTG GAA AAC TTC CTG GAA CGT CTG AAA ACC ATC ATG CGT GAA AAA 1536
Thr Leu Glu Asn Phe Leu Glu Arg Leu Lys Thr Ile Met Arg Glu Lys
            500             505             510

TAC TCT AAA TGT TCT TCC TGAGAGCTCA GTACTAGCCC GCCTAATGAG          1584
Tyr Ser Lys Cys Ser Ser
            515

CGGGCTTTTT TTTAGGCCTA                                             1604
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- 28 -

<u>Claims</u>

1.   Use of a molecule which a) specifically binds
under physiological conditions to an interleukin-4 (IL-4)
receptor expressed on a peripheral blood mononuclear cell
5   (PBMC) of an animal, and b) is capable of decreasing the
viability of said PBMC in the preparation of a medicament
for desensitizing an animal to an allergen.

2.   The use of claim 1, wherein said molecule
kills said PBMC following binding to said IL-4 receptor.

10   3.   The use of claim 1, wherein said molecule is a
hybrid molecule comprising a first and a second portion
joined together covalently, said first portion comprising
a moiety capable of decreasing cell viability and said
second portion comprising a moiety capable of
15   specifically binding to said IL-4 receptor under
physiological conditions.

4.   The use of claim 3, wherein said second
portion comprises all or a binding portion of an antibody
specific for said IL-4 receptor.

20   5.   The use of claim 3, wherein said second
portion comprises all or a binding portion of IL-4.

6.   The use of claim 3, wherein said first portion
comprises an enzymatically-active segment of a
polypeptide toxin.

- 29 -

7. The use of claim 6, wherein said polypeptide
toxin is diphtheria toxin, *Pseudomonas* exotoxin A, ricin,
Shiga toxin, Shiga-like toxin-I, Shiga-like toxin II,
Shiga-like toxin II$_v$, *E. coli* LT, *Salmonella* LT, cholera
5 toxin, C3 toxin, pertussis toxin, tetanus toxin, abrin,
modeccin, volkensin, viscumin, alorin, saporin, or
gelonin.

8. The use of claim 7, wherein said polypeptide
toxin is diphtheria toxin.

10   9. The use of claim 6, wherein said segment does
not possess a generalized eukaryotic cell-binding
activity.

10. The use of claim 9, wherein said segment
comprises fragment A of diphtheria toxin and a portion of
15 fragment B of diphtheria toxin.

11. The use of claim 10, wherein said segment
comprises DAB$_{389}$.

12. The use of claim 11, wherein said molecule is
DAB$_{389}$IL-4.

20   13. The use of claim 10, wherein said second
portion comprises an IL-4-receptor-binding portion of an
antibody specific for said IL-4 receptor.

14. Use of a molecule which is capable of
specifically binding to an IL-4 receptor expressed on the
25 surface of a B cell, and which is capable of decreasing
the viability of said B cell in the preparation of a

- 30 -

medicament for inhibiting the antibody heavy-chain class
switching of a B cell.

        15.   The use of claim 14, wherein said molecule is
a hybrid molecule comprising a first and a second portion
5   joined together covalently, said first portion comprising
a moiety capable of decreasing cell viability and said
second portion comprising a moiety capable of
specifically binding to said IL-4 receptor under
physiological conditions.

10   16.   The use of claim 15, wherein said second
portion comprises all or a binding portion of an antibody
specific for said IL-4 receptor.

        17.   The use of claim 15, wherein said second
portion comprises all or a binding portion of IL-4.

15   18.   The use of claim 15, wherein said first
portion comprises an enzymatically-active segment of a
polypeptide toxin.

        19.   The use of claim 18, wherein said polypeptide
toxin is diphtheria toxin, *Pseudomonas* exotoxin A, ricin,
20   Shiga toxin, Shiga-like toxin-I, Shiga-like toxin II,
Shiga-like toxin II$_v$, *E. coli* LT, *Salmonella* LT, cholera
toxin, C3 toxin, pertussis toxin, tetanus toxin, abrin,
modeccin, volkensin, viscumin, alorin, saporin, or
gelonin.

25   20.   The use of claim 19, wherein said polypeptide
toxin is diphtheria toxin.

- 31 -

21. The use of claim 20, wherein said segment comprises fragment A of diphtheria toxin and a portion of fragment B of diphtheria toxin.

22. The use of claim 21, wherein said segment
5 comprises DAB_{389}.

23. The use of claim 22, wherein said molecule is $DAB_{389}IL-4$.

24. The use of claim 1, further comprising the use of a second molecule which a) specifically binds
10 under physiological conditions to an interleukin-2 (IL-2) receptor expressed on said PBMC or a second PBMC of said animal, and b) is capable of decreasing the viability of the PBMC to which it binds in the preparation of said medicament.

15   25. The use of claim 1, further comprising the use of a second molecule which a) specifically binds under physiological conditions to an interleukin-6 (IL-6) receptor expressed on said PBMC or a second PBMC of said animal, and b) is capable of decreasing the viability of
20 the PBMC to which it binds in the preparation of said medicament.

1/8

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        10        20        30        40
        |         |         |         |
ATG GGC GCT GAT GTT GAT TCT TCT AAA TCT TTT GTG ATG
MET Gly Ala Asp Val Asp Ser Ser Lys Ser Phe Val MET

        50        60        70        80        90
        |         |         |         |         |
GAA AAC TTT TCT TCG TAC CAC GGG ACT AAA CCT GGT TAT GTA GAT
Glu Asn Phe Ser Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp

        100       110       120       130
        |         |         |         |
TCC ATT CAA AAA GGT ATA CAA AAG CCA AAA TCT GGT ACA CAA GGA
Ser Ile Gln Lys Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly

        140       150       160       170       180
        |         |         |         |         |
AAT TAT GAC GAT GAT TGG AAA GGG TTT TAT AGT ACC GAC AAT AAA
Asn Tyr Asp Asp Asp Trp Lys Gly Phe Tyr Ser Thr Asp Asn Lys
```

FIG. 1
(PAGE 1 OF 8)

TAC GAC GCT GCG GGG TAC TCT GTA GAT AAT GAA AAC CCG CTC TCT
Tyr Asp Ala Ala Gly Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser
190   200   210   220

GGA AAA GCT GGA GGC GTG GTC AAA GTG ACG TAT CCA GGA CTG ACG
Gly Lys Ala Gly Gly Val Val Lys Val Thr Tyr Pro Gly Leu Thr
230   240   250   260   270

AAG GTT CTC GCA CTA AAA GTG GAT AAT GCC GAA ACT ATT AAG AAA
Lys Val Leu Ala Leu Lys Asp Asn Ala Glu Thr Ile Lys Lys Lys
280   290   300   310

FIG. 1

(PAGE 2 OF 8)

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      320       330       340       350       360
      |                   |         |         |
GAG   TTA   GGT   TTA   AGT   CTC   ACT   GAA   CCG   TTG   ATG   GAG   CAA   GTC   GGA
Glu   Leu   Gly   Leu   Ser   Leu   Thr   Glu   Pro   Leu   MET   Glu   Gln   Val   Gly

            370       380       390       400
            |         |         |         |
ACG   GAA   GAG   TTT   ATC   AAA   AGG   TTC   GGT   GAT   GGT   GCT   TCG   CGT   GTA
Thr   Glu   Glu   Phe   Ile   Lys   Arg   Phe   Gly   Asp   Gly   Ala   Ser   Arg   Val

      410       420       430       440       450
      |         |         |         |         |
GTG   CTC   AGC   CTT   CCC   TTC   GCT   GAG   GGG   AGT   TCT   AGC   GTT   GAA   TAT
Val   Leu   Ser   Leu   Pro   Phe   Ala   Glu   Gly   Ser   Ser   Ser   Val   Glu   Tyr

      460       470       480       490
      |         |         |         |
ATT   AAT   AAC   TGG   GAA   CAG   GCG   AAA   GCG   TTA   AGC   GTA   GAA   CTT   GAG
Ile   Asn   Asn   Trp   Glu   Gln   Ala   Lys   Ala   Leu   Ser   Val   Glu   Leu   Glu

      500       510       520       530       540
      |         |         |         |         |
ATT   AAT   TTT   GAA   ACC   CGT   GGA   AAA   CGT   GGC   CAA   GAT   GCG   ATG   TAT
Ile   Asn   Phe   Glu   Thr   Arg   Gly   Lys   Arg   Gly   Gln   Asp   Ala   MET   Tyr
```

FIG. 1
(PAGE 3 OF 8)

GAG TAT ATG GCT CAA GCC TGT GCA GGA AAT CGT GTC AGG CGA TCA
Glu Tyr MET Ala Gln Ala Cys Ala Gly Asn Arg Val Arg Arg Ser

550   560   570   580   630

GTA GGT AGC TCA TTG TCA TGC ATA AAT CTT GAT TGG GAT GTC ATA
Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp Val Ile

590   600   610   620

AGG GAT AAA ACT AAG ACA AAG ATA GAG TCT TTG AAA GAG CAT GGC
Arg Asp Lys Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His Gly

640   650   660   670

CCT ATC AAA AAT AAA ATG AGC GAA AGT CCC AAT AAA ACA GTA TCT
Pro Ile Lys Asn Lys MET Ser Glu Ser Pro Asn Lys Thr Val Ser

680   690   700   710   720

FIG. 1
(PAGE 4 OF 8)

GAG GAA AAA CAA TAC CTA GAA GAA TTT CAT CAA ACG GCA
Glu Glu Lys Gln Tyr Leu Glu Glu Phe His Gln Thr Ala
        730   740   750   760

TTA GAG CAT CCT GAA TTG TCA GAA CTT AAA ACC GTT ACT GGG ACC
Leu Glu His Pro Glu Leu Ser Glu Leu Lys Thr Val Thr Gly Thr
        770   780   790   800   810

AAT CCT GTA TTC GCT GGG GCT AAC TAT GCG GCG TGG GCA GTA AAC
Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln
        820   830   840   850

GTT GCG CAA GTT ATC GAT AGC GAA ACA GCT GAT AAT TTG GAA AAG
Asn Pro Val Val Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys
        860   870   880   890   900

ACA ACT GCT CTT TCG ATA CTT CCT GGT ATC GGT AGC GTA ATG
Thr Thr Ala Leu Ser Ile Leu Pro Gly Ile Gly Ser Val MET
        910   920   930   940

FIG. 1
(PAGE 5 OF 8)

950

GGC AAT GCA GAC GGT GCC GTT CAC CAC AAT ACA GAA GAG ATA GTG
Gly Asn Ala Asp Gly Ala Val His His Asn Thr Glu Glu Ile Val
960   970   980   990

GCA CAA TCA ATA GCT TTA TCG TCT TTA ATG GTT GCT CAA GCT ATT
Ala Gln Ser Ile Ala Leu Ser Ser Leu MET Val Ala Gln Ala Ile
1000   1010   1020   1030

CCA TTG GTA GGA GAG CTA GTT GAT ATT GGT TTC GCT GCA TAT AAT
Pro Leu Val Gly Glu Leu Val Asp Ile Gly Phe Ala Ala Tyr Asn
1040   1050   1060   1070   1080

TTT GTA GAG AGT ATT ATC AAT TTA TTT CAA GTA GTT CAT AAT TCG
Phe Val Glu Ser Ile Ile Asn Leu Phe Gln Val Val His Asn Ser
1090   1100   1110   1120

FIG. 1
(PAGE 6 OF 8)

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                                                              1160  SphI  1170
          1130            1140            1150                 AAA ACG CAT GCT CAC
TAT AAT CGT CCC GCG TAT TCT CCG GGT CAC                       Lys Thr His Ala His
Tyr Asn Arg Pro Ala Tyr Ser Pro Gly His

                                                        1200               1210
          1180            1190                           GAA ATC ATC AAA ACT CTG AAT TCC
AAA TGC GAC ATC ACC CTG CAG                             Glu Ile Ile Lys Thr Leu Asn Ser
Lys Cys Asp Ile Thr Leu Gln

                  1230                                  1250               1260
          1220                                          GAA CTG ACG GTA ACC GAC
CTG ACC GAA CAG AAA ACT                                 Glu Leu Thr Val Thr Asp
Leu Thr Glu Gln Lys Thr

                  1280                                  1290
          1270                                          GAA AAA GAA ACC TTC TGC
ATC TTC GCA TCC AAA AAC ACC ACT                         Glu Lys Glu Thr Phe Cys
Ile Phe Ala Ser Lys Asn Thr Thr

                  1320                                  1340               1350
          1310                                          CAC CAC GAA AAA
CGT GCA GCA ACT GTT CTG CGT CAG TTC TAC TCC             His His Glu Lys
Arg Ala Ala Thr Val Leu Arg Gln Phe Tyr Ser

                  1370                                  1390
          1360                                          CAG TTC CAC CGT CAC
GAC ACT CGC TGC CTT GGT GCT ACT GCA                     Gln Phe His Arg His
Asp Thr Arg Cys Leu Gly Ala Thr Ala
```

FIG. 1
(PAGE 7 OF 8)

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      1400       1410       1420       1430       1440
       |          |          |          |          |
AAA CAG CTG ATC CGT TTC CTG AAA CGT CTA GAC CGC AAC CTG TGG
Lys Gln Leu Ile Arg Phe Leu Lys Arg Leu Asp Arg Asn Leu Trp

      1450       1460       1470       1480
       |          |          |          |
GGC CTG GCT GGC CTG AAC TCC TGT CCG GTT AAA GAA GCT AAC CAG
Gly Leu Ala Gly Leu Asn Ser Cys Pro Val Lys Glu Ala Asn Gln

      1490       1500       1510       1520       1530
       |          |          |          |          |
TCG ACC CTG GAA AAC TTC CTG GAA CGT CTG AAA ACC ATC ATG CGT
Ser Thr Leu Glu Asn Phe Leu Glu Arg Leu Lys Thr Ile MET Arg

      1540       1550
       |          |
GAA AAA TAC TCT AAA TGT TCT TCC
Glu Lys Tyr Ser Lys Cys Ser Ser
```

FIG. 1
(PAGE 8 OF 8)

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 45/05, 39/00, 37/02
US CL :424/85.1, 85.2, 85.8, 88; 514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1, 85.2, 85.8, 88; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DATABASES: US PTO-PAS, Medline
SEARCH TERMS: Interleukin-4, -6; Allergy; IgM, Immunoglobulin, Switch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences of the USA, Volume 86, issued June 1989, M. Ogata et al., "Cytotoxic activity of a recombinant fusion protein between interleukin 4 and Pseudomonas exotoxin", pages 4215-19, especially pages 4215 and 4219.	1-25
Y	European Journal of Immunology, Volume 20, issued 1990, J. C. Prinz et al., "Allergen-directed expression of Fc receptors for IgE (CD23) on human T lymphocytes is modulated by interleukin 4 and interferon-gamma", pages 1259-64, especially pages 1259 and 1263.	1-13, 24, 25

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 April 1993	**30 APR 1993**

Name and mailing address of the ISA/US	Authorized officer
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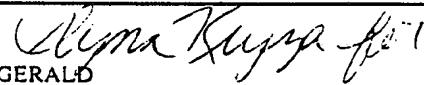
Form PCT/ISA/210 (second sheet)(July 1992)

International application No.

PCT/US93/01034

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EMBO Journal, Volume 8, Number 2, issued 1989, C. Esser et al., "Rapid induction of transcription of unrearranged s-gamma 1 switch regions in activated murine B cells by interleukin 4", pages 483-88, especially the abstract.	14-23
Y	Kidney International, Volume 35, issued 1989, T. B. Strom et al., "Toward more selective therapies to block undesired immune responses", pages 1026-33, see the entire document.	24, 25
Y	Proceedings of the National Academy of Sciences of the USA, Volume 85, issued December 1988, C. B. Siegall et al., "Cytotoxic activity of an interleukin 6-<u>Pseudomonas</u> exotoxin fusion protein on human myeloma cells", pages 9738-42, especially the abstract.	25